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The Association of Perfluoroalkyl Substances Exposure and Metabolic Syndrome in U.S. Adults

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Table of Contents

Abstract

Background: Perfluoroalkyl substances (PFASs) are persistent synthetic compounds that have been environmental contaminants since the 1950s. Exposure in the general population can be through food and water ingestion, use of non-stick cookware, and dust from stain-resistant carpet. Studies have suggested PFASs act as endocrine disrupters and to affect liver and immune function, as well as cause increases in serum lipid levels.

Objective: To explore the association between six common detectable PFASs and the metabolic syndrome (MetS) in United States (U.S.) adults.

Methods: Data from 739 participants aged 20 years and older from the 2013-2014 National Health and Nutrition Examination Survey (NHANES) were analyzed. Descriptive analysis was performed on the dataset. Univariate and multivariable logistic regression analysis was used to determine independent association between the serum PFASs and MetS controlling for confounders including age, ethnicity, income, smoking status, and gender.

Results: In the descriptive analysis, the overall prevalence of MetS in the sample population (49.5%) was higher than previously reported in the literature for U.S. adults, but distribution remained relatively equal between males (48.9%) and females (50.1%). In unadjusted logistic regression analysis, each of the six PFASs showed a positive association with MetS, but only PFOS-branched ($p < .001$) and PFHxS ($p = .019$) were statistically significant. However, after controlling for confounders, no significant association between the presence of PFASs and MetS was noted.

Conclusion: In U.S. adults aged 20 and older, current serum concentrations of PFASs are not significantly associated with MetS.

Keywords: perfluoroalkyl substances, NHANES, public health, metabolic syndrome

The Association of PFASs Exposure and Metabolic Syndrome in U.S. Adults

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a collection of persistent and stable synthetic compounds which were first manufactured in the 1950s (Lin, Chen, Lin, & Lin, 2009). Due to their heat stability and water and stain-proofing, their use became very wide-spread in consumer products. PFASs can be found in non-stick cookware, stain-resistant fabrics and carpets, waterproofing for fabrics, firefighting materials (firefighting foams), and many other areas of industry due to their ability to reduce friction.

The United States (U.S.) Environmental Protection Agency (EPA) considers PFASs as emerging contaminants based on published research, suggesting human health and environmental health concerns. Since PFASs are stable, they are very persistent in the environment (Lau et al., 2007). This also means that since their natural breakdown is slow and their presence in the environment is high due to lack of historic regulation, they pose a risk of bioaccumulation in animals, and they can enter the human food supply. Water supply contamination and transfer from food packaging and cookware into food are also potential routes of human exposure.

Since the year 2000, the EPA has been working with industry to phase out the use of PFASs (specifically PFOA and PFOS) in order to reduce the environmental burden and human exposure. Previous studies using the National Health and Nutrition Examination Survey (NHANES) data have shown that 97% of the U.S. population had detectable levels of PFASs in their serum (Hu et al., 2016). These blood levels appear to be trending down in the general population in recent years, likely due directly to the efforts by the EPA to limit its use in manufacturing (Fitz-Simon et al., 2013).

PFASs remain in the human body for years before they can be fully excreted. The half-lives for PFASs range from two to nine years (Lau et al., 2007). Health studies in humans and animals suggest that PFASs are endocrine disruptors (can affect metabolism), have a negative effect on the immune system (which can lead to cancer), cause organ dysfunction (liver/pancreas) and can cause developmental problems in offspring (Lau et al., 2007).

Metabolic syndrome (MetS), a group of risk factors that predisposes to cardiovascular disease (CVD), is characterized by hypertension, hyperglycemia, hypertriglyceridemia, reduced high-density lipoprotein (HDL) levels, and abdominal obesity (Huang, 2009; Moore, Chaudhary, & Akinyemiju, 2017). MetS prevalence in the U.S. is approximately 33% (Aguilar, Bhuket, Torres, Liu, & Wong, 2015), which is higher than the worldwide prevalence of approximately 25% (Nolan, Carrick-Ranson, Stinear, Readings, & Dalleck, 2017).

Research Question

What impact do PFASs (perfluoroalkyl and polyfluoroalkyl substances) exposure have on the development of MetS among the adult population (≥ 20 years) in the U.S.? Since PFASs have been shown to interfere with endocrine activity in humans and increase cholesterol levels, we hypothesize that there will be an association between detectable PFASs levels in serum and the presence of MetS in adults aged 20 years and greater within the U.S.

Literature Review

Metabolic Syndrome

Metabolic Syndrome (MetS) is a clustering of risk factors for developing atherosclerotic cardiovascular disease and is composed of: elevated waist circumference, elevated triglycerides (TGs), reduced HDL, elevated blood pressure, and elevated fasting glucose (Huang, 2009; Moore et al., 2017). In 2001, the National Cholesterol Education Program (NCEP) Adult

Treatment Plan (ATP) III devised criteria for defining MetS in the adult population (Table 1). The criteria are similar to the World Health Organization (WHO) criteria from 1998 and the European Group for the Study of Insulin Resistance (EGIR) of 1999, but it does not require any one criterion, as the previous definitions had. Instead it uses easily obtained laboratory and clinical measurements that clinical physicians worldwide can acquire and are simple and easy to remember (Huang, 2009). The specific criteria for MetS are any three of the five modified NCEP ATP III criteria noted in Table 1 (Department of Health and Human Services [DHHS], Centers for Disease Control and Prevention [CDC], 2009, 2009). According to guidelines from the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA), metabolic syndrome is diagnosed when a patient has at least three of the five conditions described in Table 1 (Grundy et al., 2005).

Perfluoroalkyl and Polyfluoroalkyl substances (PFASs)

Due to the manufacturing boom following the end of World War II, the environment in which humans live, work, and play has undergone many changes. This has increased human exposures to many chemicals and compounds not previously known to man, and our understanding of the long-term health risks of these exposures is not always clear (Stubleski et al., 2016). Some of these chemicals have been shown to act as endocrine disruptors in humans and a growing hypothesis is that these chemicals may be to blame in part for the increasing prevalence of MetS (Nelson, Hatch, & Webster, 2010). One such class of chemicals is PFASs. PFASs have been widely used in commercial and industrial applications since the 1950s due to their stability, water and oil repellency, and stain resistant nature. The main PFASs congeners are: perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) (Stubleski et al., 2016).

Table 1

*NCEP ATP III Criteria for Metabolic Syndrome (2005 revision)**

Risk Factor	Defining Level
Waist Circumference	
Men	> 102 cm (> 40 in)
Women	> 88 cm (> 35 in)
Blood Pressure**	≥ 130/≥85 mmHg
HDL Cholesterol**	
Men	< 40 mg/dL
Women	< 50 mg/dL
Triglycerides**	≥ 150 mg/dL
Fasting Glucose**	≥ 100 mg/dL

* According to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) at least 3 of the 5 risk factors need to be present

** Reporting to take a medication to control a risk factor counts as a having that risk factor.

Exposure to PFASs

Human exposure to PFASs is broad, since these chemicals have been used throughout industry for more than 65 years and due to their stable nature, have become ubiquitous in the environment. Industrial sites, military fire training areas, and wastewater treatment facilities have all been noted to be areas of growing concern for environmental contamination (Hu et al., 2016). All routes of exposure are not known, but the most common routes of exposure are through diet (from food packaging, the use of non-stick cookware, or possibly through bioaccumulation in food sources), contaminated drinking water sources, and household dust via particulates from stain-resistant carpeting (Nelson et al., 2010; Stubleski et al., 2016). Once in the human body, PFASs haven't been shown to undergo biotransformation. Unlike other chemical contaminants PFASs are not stored in fatty tissue. Instead, they bind to plasma proteins and are slowly excreted through renal clearance. Elimination half-lives for each

chemical differ and are as follows: PFOA has the shortest at approximately 3.8 years, PFOS at approximately 5.4 years, and PFHxS the longest at 8.5 years (DHHS CDC, 2009).

PFASs and Endocrine Function

Due to the structural similarities between PFASs and fatty acids, PFASs have been shown to bind to peroxisome proliferator-activated receptors (PPARs), which have a role in lipid metabolism and adipogenesis (Nelson et al., 2010). With evidence that PFASs bind to PPARs and previous studies showing that PFASs exert influence as endocrine-disruptors, it is conceivable that there may be an association between human serum PFASs concentrations and the prevalence of metabolic syndrome in the U.S. adult population. Most of the existing epidemiological studies report a positive association between PFASs and lipids. Evidence from general population and occupational settings suggests that PFOA may be associated with increased low-density lipoprotein (LDL) and total cholesterol levels (Steenland et al., 2010).

Evidence regarding PFASs and adverse cardiometabolic health outcomes is emerging. Among U.S. adults, serum PFOA levels were associated with higher peripheral artery disease and self- reported CVD (Shankar, Xiao, & Ducatman, 2012); this study did not assess other PFASs.

Evidence from laboratory studies has revealed that PFASs exposure is related to oxidative stress and endothelial dysfunctions which are regarded subclinical risk factors for cardiovascular and metabolic diseases (Lau et al., 2007). In human studies, although health effects of PFASs were inconsistent, a positive association between PFOA, PFOS, PFNA and total cholesterol, low density cholesterol, and poor glucose homeostasis has been noted (Lau et al., 2007; Lin et al., 2009; Fitz-Simons et al., 2013; Liu, Wen, Chu, & Lin, 2018). These are antecedents to cardiometabolic pathology.

Methods

Data Source and Study Sample

Data for 2013-2014 were obtained from the freely available National Health and Nutrition Examination Survey (NHANES), which is a population-based survey. NHANES collects health and nutritional information about noninstitutionalized U.S. individuals every two years. The survey's goal is to collect a representative sample of the U.S. population. All survey operations manuals, brochures and consent documents for the 2013-2014 dataset are publicly available on the NHANES website as part of the CDC.

The total NHANES population sample for 2013-2014 was 9,813 non-institutionalized U.S. civilians that were consented, interviewed and had examinations performed at 30 different mobile study sites throughout the U.S. Participants were sent to a mobile examination center (MEC) where laboratory measurements, physical assessments, and examinations were conducted. Laboratory testing of collected samples was conducted by the Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, GA (CDC NCHS, 2018).

From the 2013-2104 NHANES dataset, individuals with available serum PFASs levels, and available data for the five components of MetS (triglycerides, HDL, glucose, waist circumference, and blood pressure) were included in the analysis. (NHANES protocol randomly analyzed serum PFASs in 1/3 of the 2013-2014 participants that were over 12 years of age.) After filtering out individuals less for age 20 years for missing values in the dataset for the MetS criteria, PFASs serum levels, and the confounders listed below, the final sample size was N = 739 (CDC NCHS, 2018).

Confounders

Socioeconomic and sociodemographic information (i.e., age, race/ethnicity, gender, smoking status, and income) were collected by trained interviewers using the in-home NHANES Family Questionnaire via the Computer- Assisted Personal interview (CAPI) system. Race/Ethnicity was reported as Hispanic, non-Hispanic White, non-Hispanic Black, and other multi- racial (referent category).

Examination and Laboratory Procedures

Height, weight, and waist circumference were measured by trained health technicians assisted by a recorder using a stadiometer for height in meters (m), a retractable steel measuring tape for waist circumference measurements in centimeters (cm), and a digital weight scale, either portable or built into the floor of the MEC, for weight in kilograms (kg) (CDC NCHS, 2015a).

All BP determinations (systolic and diastolic) were taken in the MEC. After the participant had rested quietly in a seated position for five, three consecutive blood pressure readings were obtained using the right arm unless there was an issue with that arm. If any of the participants had any issues with both arms (like rashes, gauze dressings, casts, edema, paralysis, tubes, open sores or wounds, withered arms, a-v shunts, radical mastectomy, or if the blood pressure cuff did not fit on the arm) they were excused from this portion of the exam (CDC NCHS, 2015b).

Triglycerides, fasting glucose, HDL-C serum levels were run on all participants bloods samples that were collected at the MEC and sent to the contracted testing lab, in this case Collaborative Laboratory Services, LLC (CDC NCHS, 2013).

Tobacco smoking status was determined objectively utilizing the serum cotinine levels obtained at the MEC, with the categories broken down as ‘Non-smoker’ (referent category)

(serum cotinine level < 1 ng/mL), 'Environmental Tobacco Smoke' exposure (serum cotinine level 1 – 10 ng/mL), and 'Smoker' (serum cotinine level > 10 ng/mL) (DHHS CDC, 2009).

Metabolic Syndrome Determination

The clinical criteria for the determination of whether a participant had MetS are listed in Table 1. These criteria are based on the 2005 NCEP ATP III update (Grundy et al., 2005). A subject was considered to meet the criteria for metabolic syndrome if they had at least three of the five criteria. The male and female participants were characterized as having MetS when they met NECP recommended gender specific criteria (Table 1), or were taking medication (lowering BP, cholesterol, blood sugar) or treatment such as insulin. The official NHANES variable names used in this analysis can be found in Appendix A.

PFASs Concentration Measured via Serum Analysis

PFASs chemicals or PFCs in previous year datasets were analyzed from the blood draw performed at the MEC. A short description of the laboratory methodology used by the laboratory is as follows: Online-solid phase extraction coupled to High Performance Liquid Chromatography-Turbo Ion Spray ionization-tandem Mass Spectrometry was used for the quantitative detection of PFAS including PFHxS, (PFNA), linear PFOA (n-PFOA), sum of branched isomers of PFOA (Sb-PFOA, branched PFOA isomers), linear PFOS (n-PFOS), and sum of perfluoromethylheptane sulfonate isomers (Sm-PFOS, monomethyl branched PFOS isomers). Briefly, after dilution with formic acid, one aliquot of 100 μL of serum was injected into a commercial column switching system allowing for concentration and chromatographic separation of the analytes. Detection and quantification were done using tandem mass spectrometry (Kuklenyik, Needham, & Calafat, 2005). The lower limit of detection (LLOD) for each PFAS was 0.10 ng/mL. If a sample had analytic results below the LLOD, an imputed value

was placed in the database for that sample by the NHANES study designers. This value was determined by using the formula (LLOD/sqrt2), which yielded a result of 0.07 ng/mL for each of the six PFASs (CDC NCHS, 2016a; CDC NCHS, 2016b).

Statistical Analysis

Data analyses were performed using the Statistical Package for the Social Science (SPSS) version 25.0 (IBM Corp, Released 2016). Analysis was presented for the overall data set and also by gender. Descriptive statistics were computed overall and across gender for continuous variables including measures of centrality (mean or median) and dispersion (standard deviation or interquartile range) (age, BMI, PFASs). Frequency distributions (number and proportion) was computed for categorical variables (age, smoking, income, and race/ethnicity) and the MetS criteria (blood pressure, serum triglycerides, HDL level, waist circumference, and fasting blood sugar levels.)

Across gender, statistical significance for continuous variables was tested using Student's 2-tailed *t*-test for normally distributed variables (age and BMI) and the Mann-Whitney U test for non-normal variables (PFASs) at the $\alpha = 0.05$ level of significance. To test statistical significance for categorical variables across gender, the chi-square test was used (ethnicity, smoking status, income). As PFASs were not distributed normally, natural log-transformation was performed.

Univariate logistic regression was performed with MetS as the outcome or dependent variable (yes/no) and each of the individual natural log-transformed PFASs concentrations as a single predictor. In the second model the model was adjusted for age. In the third model, the data was adjusted for age plus income levels, ethnicity, and smoking status. In the final model, we adjusted for all available covariates including age, race/ethnicity, smoking (serum cotinine),

age, annual household income and added gender as the final variable. The Odds Ratio (OR) and 95% Confidence Interval (CI) of risk of MetS per unit change in log-transformed serum PFASs, with the two-tailed *p*-value used at an $\alpha = 0.05$ significance level. A forest plot was generated in Microsoft Excel to show the OR and 95% CI each of the six PFASs in unadjusted and multivariable adjusted models.

Results

Descriptive Statistics

The descriptive statistics of our sample aged 20 years or greater are reported in Table 2, overall ($N = 739$) and by gender. The mean age in both genders was comparable (males: 49.7 years, females: 49.9 years). The gender distribution in this sample population was relatively equal, with 48.4% male and 51.6% female. Race distribution was predominantly non-Hispanic White at 43.8%, and the predominant household annual income was > $55,000 at 38.2%. Smoking status, revealed that 72.3% were non-smokers, 23.5% were smokers, and 4.2% were exposed to environmental tobacco smoke. The mean BMI for both males and females fell within the 'overweight' category with mean values of 28.4 for males and 29.4 for females. Females in this sample were slightly heavier as compared to males and had an average 1.97 points higher BMI than their male counterparts. This difference was statistically significant ($p = .039$).

The prevalence of MetS was 49.5%, and nearly evenly distributed between males (48.9%) and females (50.1%). In this population, 10.6% used diabetes medications or insulin, 26% used cholesterol medications, and 31.4% used blood pressure medications. When comparing of the individual MetS criteria by gender, more males met MetS criteria for elevated triglycerides, and higher fasting blood glucose. In contrast, more females met the MetS criteria for larger waist circumference and low HDL; however, both males and females had relatively

equal distribution of high blood pressure defined by MetS criteria. PFASs levels obtained from the NHANES 2013-2014 dataset were analyzed as shown in Table 2. Males had higher serum PFASs concentrations compared to females, which was statistically significant in branched PFOS ($p < .001$), linear PFOA ($p = .001$), PFHxS ($p < .001$) and PFNA ($p = .014$). However, linear PFOS levels were not significantly different between males and females.

Table 2

Characteristics of 2013-2014 NHANES Adult Participants, Distribution of Serum PFAS Levels, and Metabolic Factors, Overall and by Gender

Characteristic Variable	Overall		Male		Female		*p*-value [a]
	n	Mean ± SD or percent	n	Mean ± SD or percent	n	Mean ± SD or percent	
Age (years)	739	49.8 ± 17.6	358	49.7 ± 17.6	381	49.9 ± 17.6	.763
BMI (kg/m^2)	737	28.9 ± 6.9	356	28.4 ± 5.7	381	29.4 ± 7.8	*.039*
Race/ethnicity							.770
Non-Hispanic White	324	43.80%	155	43.30%	169	44.40%	
Non-Hispanic Black	150	20.30%	79	22.10%	71	18.60%	
Hispanic	155	21.00%	73	20.40%	82	21.50%	
Asian	87	11.80%	39	10.90%	48	12.60%	
Other	23	3.10%	12	3.40%	11	2.90%	
Annual Household Income							.121
< $25,000	226	32.50%	98	29.00%	128	35.80%	
$25,000 to $54,999	204	29.30%	100	29.60%	104	29.10%	
≥ $55,000	266	38.20%	140	41.40%	126	35.20%	
Smoking status [c]							.157
Non-smoker	534	72.30%	247	69%	287	75.30%	
ETS	31	4.20%	17	4.70%	14	3.70%	
Smoker	174	23.50%	94	26.30%	80	21%	
Metabolic Syndrome [e]	366	49.50%	175	48.90%	191	50.10%	
BP ≥130/≥90 mmHg [g]	343	46.40%	171	47.80%	172	45.10%	
TG ≥ 150 mg/dL [g]	331	44.80%	170	47.50%	161	42.30%	
FBG ≥ 100 mg/dL [g]	375	50.70%	209	58.40%	166	43.60%	
WC elevated [g]	418	56.60%	154	43.00%	264	69.30%	
HDL low [g]	358	48.40%	161	45.00%	197	51.70%	
Medications [d]							
Diabetes Medications	78	10.60%	43	12.00%	35	9.20%	
Cholesterol Medications	192	26.00%	95	26.50%	97	25.50%	
BP Medications	232	31.40%	101	28.20%	131	34.40%	
Polyfluoroalkyl Substances [b]		Median (IQR)		Median (IQR)		Median (IQR)	
PFOS - linear (ng/mL)[f]	739	3.8 (4.7)	358	4.5 (5.03)	381	2.9 (4.0)	.123
PFOS - branched (ng/mL)[f]	739	1.6 (2.1)	358	2.2 (1.9)	381	1.1 (1.6)	*<.001*
PFOA - linear (ng/mL)[f]	739	1.9 (1.8)	358	2.2 (1.5)	381	1.6 (1.8)	*.001*
PFOA - branched (ng/mL)[f]	739	0.07 (0)	358	0.07 (0)	381	0.07 (0)	.866
PFHxS (ng/mL)[f]	739	1.4 (1.7)	358	1.8 (1.6)	381	1.0 (1.3)	*<.001*
PFNA (ng/mL)[f]	739	0.7 (0.6)	358	0.8 (0.8)	381	0.6 (0.5)	*.014*

Note: ETS, environmental tobacco smoke. HDL, High Density Lipoproteins. FBG, fasting blood glucose. WC, waist circumference. BP, blood pressure. TG, triglycerides.

[a] *p*-Value tests differences between males and females using the chi-square test for categorical variables, t-tests (without assuming equal variances) for variables for which the mean is presented, and Mann-Whitney tests for variables for which the median is presented. [b] Untransformed serum perfluoroalkyl substances; Not normally distributed, Median and (IQR) reported. [c] Smoking categories based on serum cotinine levels. [d] Actively taking medications counts as criteria for metabolic syndrome for BP, TG, HDL, and FBG. [e] Met 3 of 5 NHEP ATP III criteria for metabolic syndrome. [f] Median (IQR; inter-quartile range). [g] For BP, HDL, FBG, and TG variables, value either in the metabolic syndrome range or on medication for issue represented.

Univariate and Multivariable Logistic Regression Analysis

As shown in Table 3, in univariate logistic regression all PFASs showed an odds ratios MetS greater than one, suggesting an increased risk of having MetS, but only two of these PFASs reached statistical significance [PFOS-branched (OR=1.32, 95% CI=1.13-1.53, $p < .001$), PFHxS (OR=1.20, 95% CI=1.03-1.39, $p < .019$)]. However, the significant associations between MetS and these two variables was lost after adjusting for confounders. Similarly, in multivariable analysis, none of the other six PFASs had statistically significant association with MetS. These results did not change after further adjustment for gender. Figures 1 and 2 illustrate the data using forest plots to show the OR and 95% CI for each of the six PFASs in unadjusted and multivariable adjusted models, respectively.

Table 3

Univariate, Age Adjusted and Multivariable Adjusted and Multivariable Plus Gender Adjusted Logistic Regression Showing Association of Log Transformed Perfluoroalkyl Substances with Metabolic Syndrome

PFAS n = 739	OR	95% CI	*p*-value
PFOA - branched			
Unadjusted[a]	1.23	0.88 - 1.71	.231
Age Adjusted[b]	0.89	0.62 - 1.28	.527
Multivariable Adjusted[c]	0.91	0.62 - 1.33	.629
Multivariable + Gender Adjusted[d]	0.91	0.62 - 1.33	.633
PFOA - linear			
Unadjusted[a]	1.09	0.90 - 1.31	.392
Age Adjusted[b]	0.81	0.66 - 1.00	*.048*
Multivariable Adjusted[c]	0.86	0.69 - 1.08	.194
Multivariable + Gender Adjusted[d]	0.85	0.68 - 1.07	.162
PFOS - branched			
Unadjusted[a]	1.32	1.13 - 1.53	< *.001*
Age Adjusted[b]	0.98	0.83 - 1.16	.827
Multivariable Adjusted[c]	0.98	0.82 - 1.17	.797
Multivariable + Gender Adjusted[d]	0.96	0.79 - 1.17	.673
PFOS - linear			
Unadjusted[a]	1.08	0.93 - 1.24	.307
Age Adjusted[b]	0.88	0.75 - 1.03	.113
Multivariable Adjusted[c]	0.89	0.75 - 1.06	.207
Multivariable + Gender Adjusted[d]	0.88	0.73 - 1.05	.162
PFNA			
Unadjusted[a]	1.09	0.89 - 1.32	.400
Age Adjusted[b]	0.80	0.64 - 1.00	*.047*
Multivariable Adjusted[c]	0.85	0.67 - 1.07	.169
Multivariable + Gender Adjusted[d]	0.84	0.66 - 1.06	.143
PFHxS			
Unadjusted[a]	1.20	1.03 - 1.39	*.019*
Age Adjusted[b]	0.92	0.78 - 1.09	.347
Multivariable Adjusted[c]	0.93	0.78 - 1.12	.440
Multivariable + Gender Adjusted[d]	0.91	0.75 - 1.11	.339

PFOA - Perfluorooctonoaic acid; PFOS - Perfluorooctane sulfonic acid; PFHxS - Perfluorohexane sulfonic acid; PFNA - Perfluorononanoic acid

[a]Odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models unadjusted. [b]Odds Ratios (OR) and 95% confidence intervals (CI) from logistic regression models adjusted for age. [c]Odds Ratios (OR) and 95% confidence intervals (CI) from logistic regression models adjusted for age, ethnicity, income levels and smoking status (derived from serum cotinine levels). [d]Odds Ratios (OR) and 95% confidence intervals (CI) from logistic regression models adjusted for age, ethnicity, income levels, smoking status (derived from serum cotinine levels), and gender.

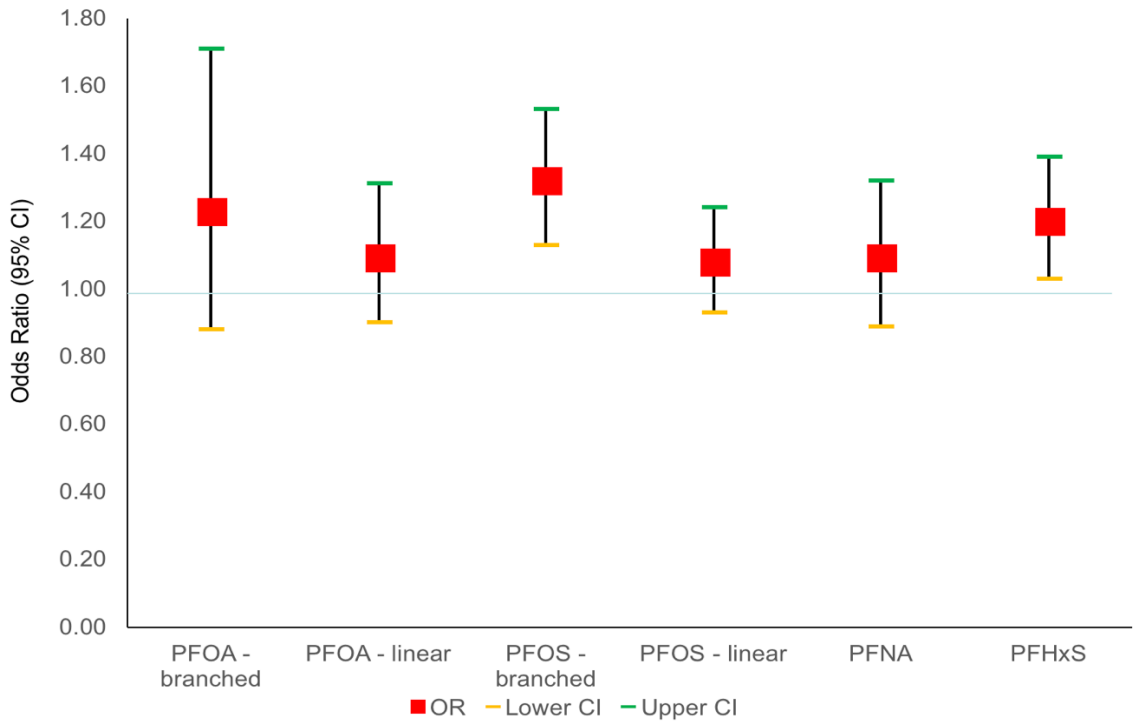

Figure 1. Unadjusted logistic regression showing association of log-transformed PFASs with metabolic syndrome.

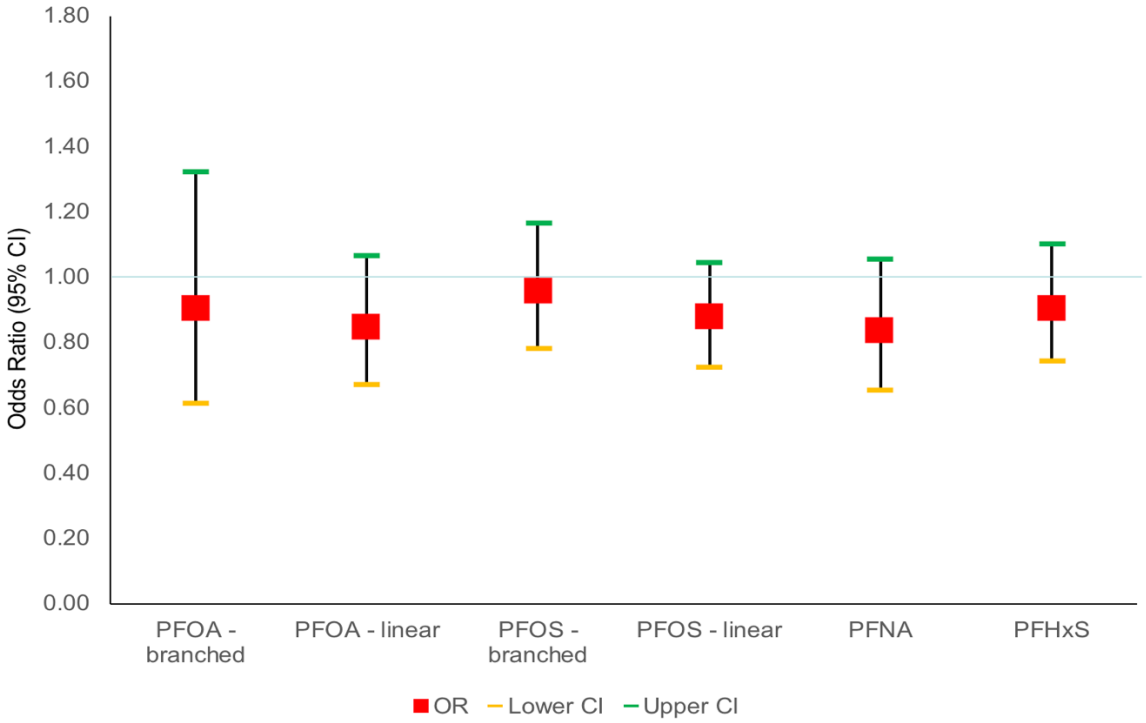

Figure 2. Multivariable adjusted logistic regression showing association of log-transformed PFASs with metabolic syndrome.

Sensitivity Analysis

A sensitivity analysis was conducted to explore if excluding participants with serum PFAS <LOD modified the results. In unadjusted models, branched PFOS and PFHxS showed a higher risk of MetS ($p < .001$ for both). In contrast, PFNA showed a lower risk ($p = .047$) of MetS in age adjusted model. However, in multivariable analysis adjusting for confounders, none of the six PFAS indicated a significant risk of MetS. Excluding participants with serum levels less than LOD for PFAS values did not change our conclusion.

Discussion

In this cross-sectional analysis of the NHANES 2013-2014 data, although all six PFASs tested had suggested there was a significant association with MetS in U.S. adults aged 20 years and older in the unadjusted models, this association was not statistically significant after adjusting for confounders.

Previous studies have shown a positive association between elevated serum lipid levels and elevated PFOA and PFOS levels, and that that those abnormal lipid levels corrected over time as the PFASs levels in their serum decreased over time with natural clearance (Fitz-Simon et al., 2013). However, the study by Fitz-Simon et al. (2013) was completed in a population with known elevated environmental exposure and not the U.S. general population exposure as explored in the current analysis.

An interesting observation in our analysis was that the prevalence of MetS in our studied population which is representative of the U.S. population, was near 50%. According to published reports prevalence of MetS in the U.S. adult population has been gradually increasing over the years, as reported in a research letter in JAMA, May 2015, which showed that the prevalence of MetS in NHANES datasets rose from 32.9% in 2003-2004, to 34.7% in 2011-2012

(Aguilar et al., 2015). In 2014, the highest MetS prevalence was 38.6% in Hispanics, 37.4% in non-Hispanic Whites, and over 50% for women and Hispanics over the age of 60 (Aguilar et al., 2015). Other recent international studies have reported a similar increasing magnitude of MetS prevalence. An overall MetS prevalence above 50% in adults over 45 years of age was noted (Delavari, Forouzanfar, Alikhani, Sharifian, & Kelishadi, 2009), which was concurrent with our findings, suggesting the worldwide prevalence of MetS is on the rise.

Although the associations seen were not statistically significant, our findings showing a negative relationship between the PFASs and MetS after adjusting for confounding variables. These results match what others have seen in the same NHANES 2013-2014 dataset suggesting the presence of PFASs may infer some protection against low HDL levels, and high triglyceride levels, although the mechanism for this is yet unknown (Liu et al.., 2018).

A sensitivity analysis was conducted to explore if excluding participants with serum PFAS <LOD modified the results. In unadjusted models, PFOS-branched and PFHxS showed a higher risk of MetS ($p < .001$ for both). In contrast, PFNA showed a lower risk ($p = .047$) of MetS in the age-adjusted model. However, in multivariable analysis, none of the six PFAS variables indicated a significant risk of MetS. The conclusion of the sensitivity analysis was that excluding those participants with serum levels of PFAS below LOD did not change the outcomes.

Strengths

The study had several strengths. Data from NHANES, which is nationally representative, characterizing both genders and ethnicities, was used. We adjusted for a number of confounding variables including lifestyle, and socioeconomic status. Earlier publications from NHANES have reported association of MetS with two PFASs isomers (PFOA, PFOS) (Liu et al., 2018).

To our knowledge this is the first analysis in which two additional congeners (PFHxS, PFNA) were assessed regarding their impact on MetS.

Limitations

Our study had several limitations. First, the cross-sectional design of the study does not allow us to infer any causation between PFAS and MetS. Second, our study did not look at the effect of higher PFASs levels and metabolic syndrome specifically. Instead, we focused on whether any presence of PFASs in the serum could have an association with metabolic syndrome. Other studies have demonstrated a positive association between higher PFASs levels and glucose homeostasis, especially in adolescents (Lin et al., 2009), but we didn't take adolescents into account for our analysis. This could be an area of future research to see if there continues to be an association between higher levels of PFASs and MetS, especially in the adult population. Also, we were unable to control for seafood intake, which is a significant source of PFASs exposure.

Conclusion

According to the results of our study, the presence of PFASs in the serum of U.S. adults aged 20 years and greater does not have a statistically significant association with the prevalence of MetS. Further study comprising of a larger dataset combining data from several survey cycles is needed to evaluate the effect of serum levels of PFASs on the prevalence of MetS.

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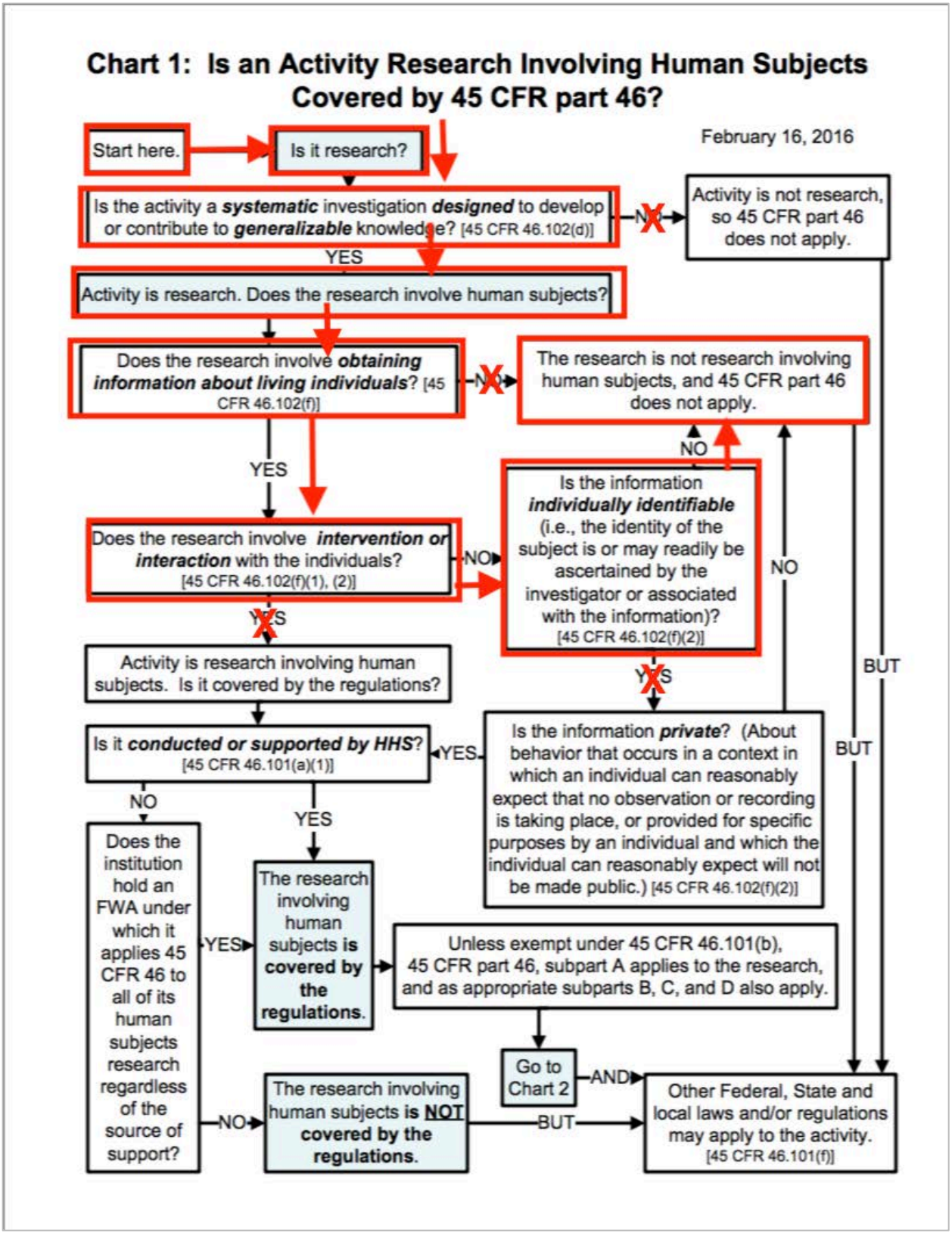
Appendix A:

NHANES 2013 – 2014 Dataset Variable Names

NHANES Variable Name	Variable	Description
BPQ40A	Hypertension Medication	Reported taking hypertension medication
BPQ050A	Hypertension Medication	Reported taking prescription for hypertension
BPQ090D	Cholesterol Medication	Told to take prescription for cholesterol
BPQ100D	Cholesterol Medication	Now taking prescription for cholesterol
DIQQ050	Diabetic Medication	Reported taking insulin
DIQ070	Diabetic Medication	Reported taking hypoglycemic medication
SSMPFOS	Serum PFAS Level	Branched isomers of perfluorooctane sulfonate (PFOS)
SSNPFOS	Serum PFAS Level	Linear isomers of perfluorooctane sulfonate (PFOS)
SSBPFOA	Serum PFAS Level	Branched isomers of perfluorooctanoate (PFOA)
SSNPFOA	Serum PFAS Level	Linear isomers of perfluorooctanoate (PFOA)
LBXPFNA	Serum PFAS Level	Perfluorononanoate (PFNA)
LBXPFHS	Serum PFAS Level	Perfluorohexane sulfonate (PFHxS)
LBXGLU	Fasting Glucose Level	Serum fasting glucose
LBXHDD	HDL Cholesterol	Serum fasting high density lipoprotein (HDL)
LBXSTR	Triglycerides	Serum fasting triglycerides
LBXCOT	Cotinine Level	Serum cotinine levels measure smoking status
RIAGENDR	Gender	Gender of the participant (male or female)
RIDAGEYR	Age	Age of participant in years
RIDRETH3	Race	Recode of reported race of participant
INDFMIN2	Income	Annual family income in US dollars
BPXSY1, BPXSY2, BPXSY3	Systolic Blood Pressure	First, second, and third reading of systolic blood pressure of participant
BPXDI1, BPXDI2, BPXDI3	Diastolic Blood Pressure	First, second, and third reading of diastolic blood pressure of participant
BMXBMI	BMI	Body Mass Index (kg/m2)
BMXWAIST	Waist Circumference	Waist circumference measurement in cm

Note: NHANES=National Health and Nutrition Examination Survey, PFAS=perfluoroalkyl substance, BMI=body mass index, cm=centimeters.
Source: Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), 2018.

Appendix B: Human Subjects Regulations Decision Chart



Appendix C - List of Competencies Met in Integrative Learning Experience

Wright State Program Public Health Competencies Checklist

Assess and utilize quantitative and qualitative data.
Apply analytical reasoning and methods in data analysis to describe the health of a community.
Describe how policies, systems, and environment affect the health of populations.
Evaluate and interpret evidence, including strengths, limitations, and practical implications.
Demonstrate ethical standards in research, data collection and management, data analysis, and communication.

Concentration Specific Competencies Checklist

Population Health Concentration
Demonstrate application of an advanced qualitative or quantitative research methodology.
Demonstrate the ability to contextualize and integrate knowledge of a specific population health issue.